EUGLENA GRACILIS IN SYNCHRONOUS DIVISION. II. BIOSYNTHETIC RATES OVER THE LIFE CYCLE

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Mapping of the major metabolic and synthetic events occurring over the course of the life cycle of single cells forms one of the principal endeavors of current physiological efforts, and very refined (albeit tedious) techniques have permitted notable successes in this area. However, the small size of growing cells imposes serious quantitative—and qualitative—limitations on the information to be gleaned from this approach, since many of the coarser techniques require masses several orders of magnitude above that provided by the single cell. Furthermore, natural variability of cells grown under identical conditions—best exemplified perhaps in the scatter of individual generation times (Powell, 1955; Prescott, 1959)—can often be extensive. The use of synchronously-dividing populations as a model of the single cell promises to surmount some of these difficulties, though new ones may well be introduced.

That populations of cells can be induced to divide rhythmically has been known for some time (von Denffer, 1949), but a clear appreciation of the possibilities offered by such systems appeared only within the last decade (Hotchkiss, 1954; James, 1954; Scherbaum and Zeuthen, 1954). It has already been made the subject of several review articles (Scherbaum, 1960; Zeuthen, 1958). Increased interest in this field has resulted in the elaboration of certain obvious restrictions which should be satisfied by the synchronously-dividing population before it may fairly be viewed as a working model of the single cell. For example, the time consumed in the burst of division activity should be relatively short compared to the over-all generation time; and the increase in population number occurring in each burst should be commensurate with the normal division behavior of member cells, if the division burst is to be equated to division of the single cell. These are but two of the more obvious parameters which characterize synchronous cell division. More subtle restrictions include continued and repetitive division bursts, *i.e.,* populations in which synchrony can be maintained indefinitely (as long as the environmental stimulus causing synchrony is continued) (Padilla and James, 1960). A first corollary of continued division synchrony is the "balanced growth" of all member cells, in the usage of Barner and Cohen (1956). A further restriction is always implied, namely that growth of member cells in the population be

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at least as synchronous as their division. Unfortunately, none of the cell types thus far synchronized can completely satisfy all these restrictions; as noted by Zeuthen (1958), however, even a modest degree of synchrony can be useful.

It is possible to synchronize division activity of free-living photosynthetic cells, at a constant temperature, merely by growing the cells autotrophically on light-dark cycles (Sweeney and Hastings, 1958: Tamiya *et al.*, 1953). If the light and dark periods are of appropriate lengths, the average cell grows uninterruptedly over one whole generation, and stores sufficient energy in the light period to satisfy demands for those processes culminating in cell division (Bernstein, 1960; Cook and James, 1960). In all photosynthetic systems so grown, the "burst" of division activity is confined for the most part to the dark period, so that growth and division are truly separated.

In populations of *Euglena gracilis,* the percentage of cells completing division in successive bursts gradually decreases with increased population density, presumably because mutual shading limits effective utilization of the photosynthetic apparatus. At population densities below about 5000 cells per ml., 90–95% of the cells complete division at each burst; if the culture is diluted once in each generation with fresh salt medium (at the temperature of the culture), so that the population density never approaches levels of the stationary phase, division synchrony can be maintained without abatement for long periods of time, perhaps indefinitely (see Figure 1). It can be seen from Figure 1 that divisions commence

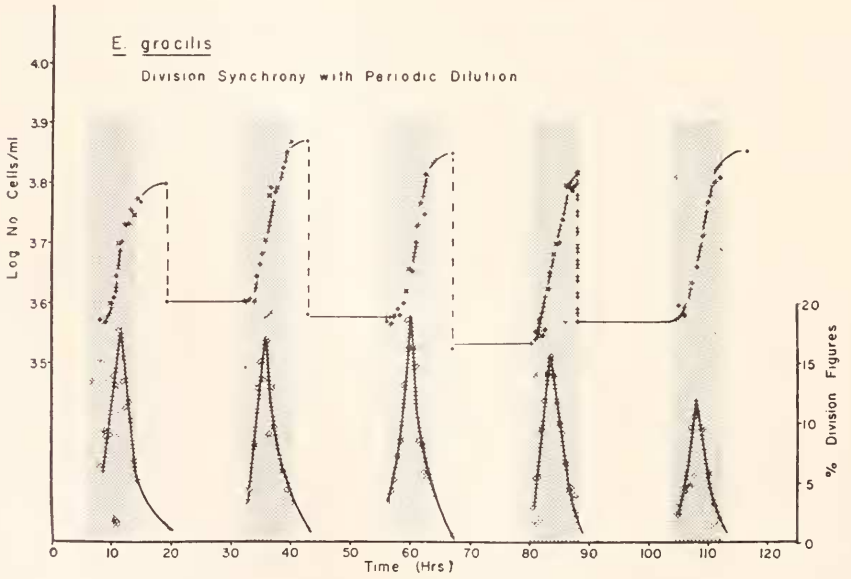

FIGURE 1. Light-induced division synchrony of *Euglena gracilis.* The upper curve is a plot of the logarithm (to the base 10) of the population density. The 8-hour dark periods, to which nearly all of the cell divisions are confined, are represented by the shaded areas. The light periods are of 16 hours duration. The broken lines indicate dilution with fresh salt medium at the temperature of the culture (20° C.). The lower curve shows the proportion of cells in recognizable fission.

at about the third hour of each dark period, and are terminated some two or three hours after the onset of the subsequent light period. Except for this very early portion, no cell divisions occur in the light period, which has a total duration of 16 hours. Of those cells completing division, 95% do so within a 6-hour span of time (James, 1960). Thus, about 75% of the total generation time is devoid of cell divisions, and most of this fraction is spent in the light, where major biosynthetic activities of *Euglena* should occur. Since the division synchrony of *Euglena gracilis* is well-defined and repetitive, the metabolic regulation of synthetic rates must be in fine adjustment at all ages, especially since the energy supplied (as light) is just that needed for growth and division of the average cell over one life cycle. Characterization of growth patterns in synchronized *Euglena* is therefore profitably made, and cultivation on a near-basal medium, where the photosynthetic apparatus itself must be duplicated, perhaps renders such a study all the more meaningful. Moreover, synchronous growth at a constant temperature obviates difficulties associated with the interpretation of temperature-sensitive rate processes, and thus circumvents some of the criticisms which have been leveled against other methods of synchronizing cell division (Abbo and Pardee, 1960; Mitchison and Walker, 1959; Prescott, 1960).

Mass increase and the distribution of volumes in member cells of synchronized *Euglena* populations have been reviewed in a previous paper, and the analogy of the population as a single cell discussed in some greater detail (Cook, 1961). While a wide range of cell volumes exists at any time, the (weighted mean) volume and dry mass exhibit a linear increase, to a doubling, over the whole of the 16-hour light period. The average *Euglena* in synchronous populations, then, is to a first approximation a successful working model of the single cell. The present report will describe growth patterns of other important cell parameters over the life cycle.

Methods and Materials

Culture methods have been described in detail previously (Cook and James, 1960). A constant-temperature water bath (American Instrument Co.) circulated water continuously through a thin transparent plastic jacket surrounding the cylindrical culture vessel, so that the culture was maintained at 20° C. An in-line reservoir of fresh medium, also kept at 20°, was used to dilute the culture once in each generation (see Figure 1), so that the population density at harvest always lay between 3000 and 5000 cells per ml., well below levels of the stationary phase. While this approach is only quasi-chemostatic, it is not likely that the medium ever became limiting for growth of *Euglena;* and in addition, changing characteristics of the average cell as a function of population density were avoided (Summers, 1960).

The medium was that of Cramer and Myers (1952), slightly modified (Padilla and James, 1960): $(NH_4)_2HPO_4$, 1.0 gm./L.; KH_2PO_4, 1.0 gm./L.; $MgSO_4 \cdot xH_2O$, 0.2 gm./L.; $CaCl_2$, 0.02 gm./L.; $Fe_2(SO_4)_3 \cdot xH_2O$, 3.0 mg./L.; $MnCl_2 \cdot 4H_2O$, 1.8 mg./L.; $CoSO_4 \cdot 7H_2O$, 1.5 mg./L.; $ZnSO_4$, 0.4 mg./L.; $Na_2MO_4 \cdot 2H_2O$, 0.2 mg./L.; $CuSO_4 \cdot 5H_2O$, 0.02 mg./L. These salts are chelated by sodium citrate (0.8 gm./L.), which is not used as a carbon source by *Euglena.* The vitamins B_1 (0.1 mg./L.) and B_{12} (0.0005 mg./L.) are required by *Euglena,* and were added aseptically to the autoclaved salt-citrate solution after sterilization by filtration. The phosphates buffer the medium to a pH of 6.8. In this medium, $(NH_4)_2HPO_4$

is the sole nitrogen source. However, small amounts of the sulfhydryl-bearing amino acids methionine (10^{-5} *M*) and cysteine hydrochloride (6.4×10^{-4} *M*) were routinely added to the medium (after sterilization by filteration). While these concentrations do not support growth, they can effectively improve division characteristics of *Euglena* in synchronous culture (Cook and James, 1960; James, 1960). Incandescent lamps (General Electric Co.), with an incident intensity of 130 foot-candles, were the sole energy source, and nutrition, save for catalytic amounts of the vitamins and two amino acids, was strictly autotrophic. In continuous light at 20° C., this intensity is saturating for growth of *Euglena*.

Cultures were continuously stirred, by means of a teflon-covered magnetic impeller bar, to ensure even illumination and rapid exchange of gases. Harvests were made by means of a gravity-flow siphon, and stirring further served to randomize the distribution of cells within the culture. After the syphon was flushed, all aliquots were representative of the population as a whole.

Cultures were axenic, and were kept on the light-dark cycle from inoculation. Dilution of the medium was initiated when the appropriate population density was reached, and repeated once in each generation; harvests of any given culture were deferred until at least five light-dark cycles had elapsed after the first dilution. Generally, two light-dark cycles suffice for the induction of division synchrony, and initial harvests were delayed only as a precaution against the possible tardy stabilization of other cellular parameters.

All cell counts were made with the Coulter cell counter (Coulter Co.). Details of this procedure can be found elsewhere (Mattern *et al.*, 1957); the mean values of replicate counts made with this instrument are within 2% of the true value when no correction for coincidence is necessary. For the present case, aliquots were always diluted to cell densities yielding a linear counting rate, so that coincidence was negligible.

At harvest, cells were first packed by gentle centrifugation, and then resuspended to densities appropriate to the analytical procedure being followed. Representative aliquots of this concentrated suspension were then diluted with the salt medium for cell counts.

Total protein content of the average cell was estimated by the Folin-Ciocalteau phenol test as modified by Lowry *et al.* (1951), after prior extraction of pigments and other soluble fractions with 90% aqueous acetone. Bovine serum albumin in known concentrations was simultaneously run as a standard, and protein levels in *Euglena* are expressed in these equivalents.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were extracted according to the method of Schmidt and Thannhauser (1945). Optical densities of the hydrolyzed DNA and RNA (in 5% perchloric acid) were measured at 260 and 315 mμ with the Beckman DU spectrophotometer. Regression curves obtained with serial concentrations of commercial DNA and RNA permitted conversion of the observed difference reading ($O.D._{260} - O.D._{315}$) into absolute amounts per cell.

Pigments of the photosynthetic apparatus were estimated according to the method of Richards with Thompson (1952). After overnight extraction of a known number of cells in 90% aqueous acetone at 15° C., the initial extract was made up to volume (10 ml.) with two washes. Optical densities were determined in the Beckman DU spectrophotometer at wave-lengths of 750, 665, 645, 630, 510,

and 480 mμ, with 90% acetone as a blank. Pigments in *Euglena* include the chlorophylls *a* and *b*, and several carotenoids, of which beta-carotene is the most important in terms of absolute amounts (Fogg, 1953). None of these absorb appreciably at 750 mμ, and readings at this wave-length (usually negligible) were routinely subtracted from those at the other wave-lengths as a correction for turbidity. Simultaneous equations developed by Richards with Thompson (1952) permit calculation of pigment concentrations from the observed optical densities. For the chlorophylls, concentrations in absolute amounts are obtained. The method serves as a satisfactory measure of chlorophyll *a* in *Euglena*. Chlorophyll *b* is present in much smaller amounts, however (about 10% that of chlorophyll *a*). This pigment is therefore largely masked by the other pigments, and accurate measurements are not feasible by this method. Since the several carotenoids are estimated as a mixture, concentrations must be expressed in arbitrary units. Richards with Thompson have proposed the Standard Pigment Unit (SPU) for this measure, and this unit is retained here. Their equations are satisfactory for estimates of carotenoid content in *Euglena* (Cook, 1960).

The method of cultivating cells which was adopted for these studies limited the working volume of the culture to about one liter per day, *i.e.*, a yield of no more than 5×10^6 cells in each generation. Since some of the procedures required almost this many cells for a single determination, a complete picture of synthetic rates could

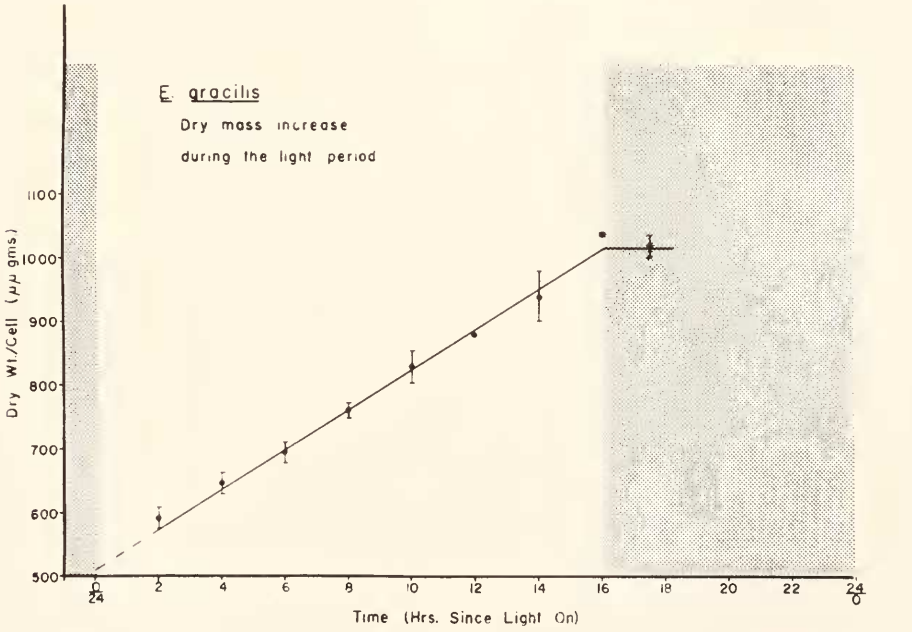

FIGURE 2. Dry mass of the average cell at different times during any given light-dark cycle. The abscissa is the time scale for one complete cycle, beginning arbitrarily with the onset of the 16-hour light period. The shaded area at the left represents the end of the preceding dark period, and that at the right the whole 8-hour dark period for the cycle shown. Limits of one standard deviation are indicated.

be compiled only by pooling data from many different light-dark cycles. Continued synchronous culture by periodic dilution, as illustrated in Figure 1, thus served practical as well as theoretical reasons. In the following, statistical estimates of experimental error are shown as the standard deviation about the mean, and represent both errors of analysis and of cell counts.

Unfortunately, the cell used in these studies was previously referred to as *Euglena gracilis* var. *bacillaris* (Cook and James, 1960). Certain characteristics, not discovered until after that paper had been accepted for publication, made it clear that the cell was not in fact *E. gracilis* var. *bacillaris,* but another strain of the species. Pending positive identification, the cell is called merely *Euglena gracilis.*

Results

Figure 2 shows the dry weight of the average *Euglena,* measured at different times during the light-dark cycle. These data have been reviewed before (Cook, 1961), and are shown here for reference. The dark period is represented by the shaded portion, and the abscissa is the time-scale for one complete cycle—24 hours—beginning arbitrarily with the onset of the light period. The rate of dry mass increase is clearly linear over the whole of the light period, and is terminated when the cells enter the dark period. Since cell divisions are still occurring in the first two hours of the light period, meaningful mass measurements on a per cell basis are not easily made at this time. It seems fair to estimate mass changes in the early hours of the light period by extrapolation from the rate observed after cell divisions are complete. The extrapolated portion of the rate curve in Figure 2 is indicated by the broken line. (This treatment ignores the possibility of a brief lag in mass accumulation very early in the light period.) Since *Euglena* essentially doubles in number at each burst of division activity, continued balanced growth demands a duplication of major cell constituents over the life cycle. Figure 2 indicates that this is indeed the case for the dry weight of *Euglena;* the average filial cell has a dry mass of about 500 $\mu\mu$grams, and the average parent cell 1000 $\mu\mu$grams. Mass loss in the 8-hour dark period is negligible.

Synthetic rates for total protein are not so straightforward. As can be seen from Figure 3, protein synthesis is terminated (after a doubling) at the tenth or eleventh hour of the light period, at a level which remains constant thereafter. The filial cells contain about 200 $\mu\mu$grams protein, and the parent cells 400. The protein : dry mass ratio is 0.4 both at the beginning and end of the light period, but goes through a slight maximum (0.5) in the interim.

Nucleic acid levels in the average cell were determined less frequently. Figure 4 shows the RNA content at four different ages. If the rate curve is again extrapolated to the beginning of the light period, it can be seen that a doubling occurs linearly during the entire light period. The filial cell contains 23 $\mu\mu$grams, and the parent cell 46 $\mu\mu$grams. Less confidence can be placed in the DNA data, and this study will bear repetition with culture conditions yielding larger numbers of cells for this analysis. However, Figure 5 indicates that DNA is probably doubled in a linear fashion throughout the light period.

Duplication of chlorophyll *a* is completed by the fourteenth hour of the light period, after which no further increase occurs (Fig. 6). Similarly, carotenoid synthesis (in *Euglena,* mostly beta-carotene) is terminated about two hours before

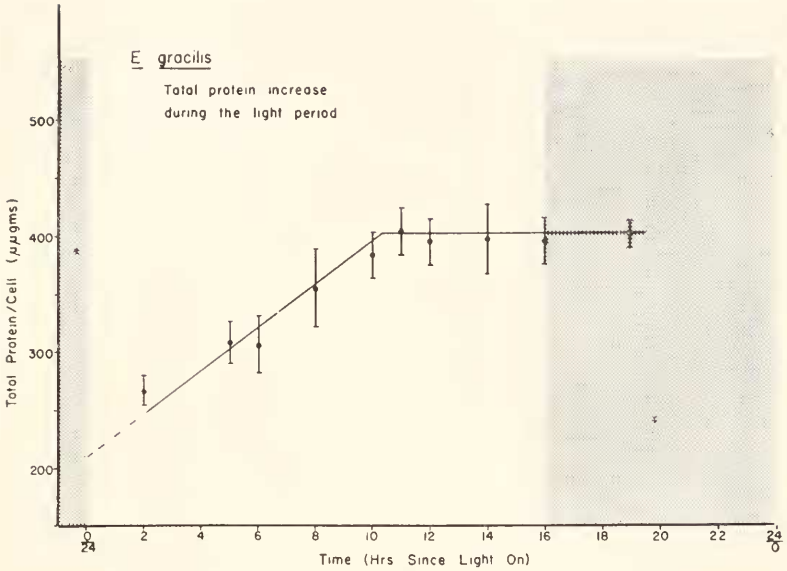

FIGURE 3. Total protein of the average *Euglena* at different times during any given light-dark cycle.

the onset of the dark period (Fig. 7). The ratio carotenoids : chlorophyll *a* is thus constant throughout the life-cycle of the average cell. Chlorophyll *a* contributes about 3% to the total dry weight of *Euglena,* although this value goes through a slight maximum at the fourteenth hour of the light period. From data of Goodwin and Jamikorn (1954), it can be calculated that the carotenoids in synchronized

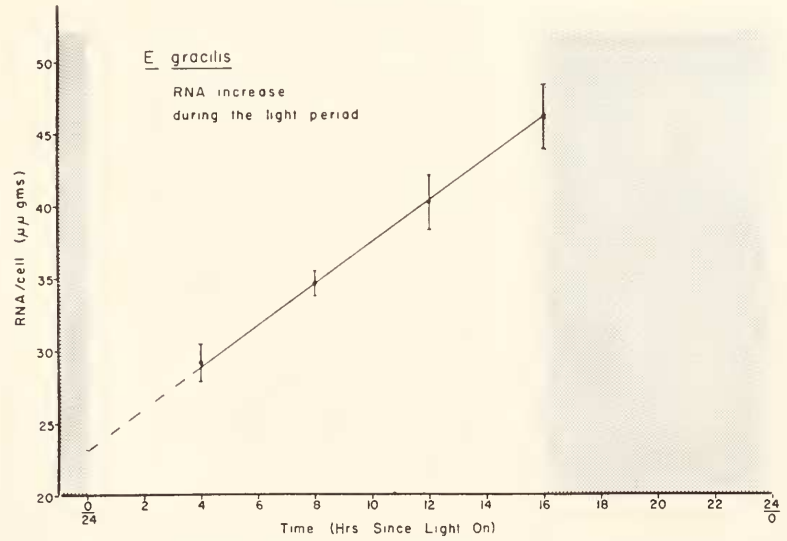

FIGURE 4. RNA synthesis by the average cell during the light period.

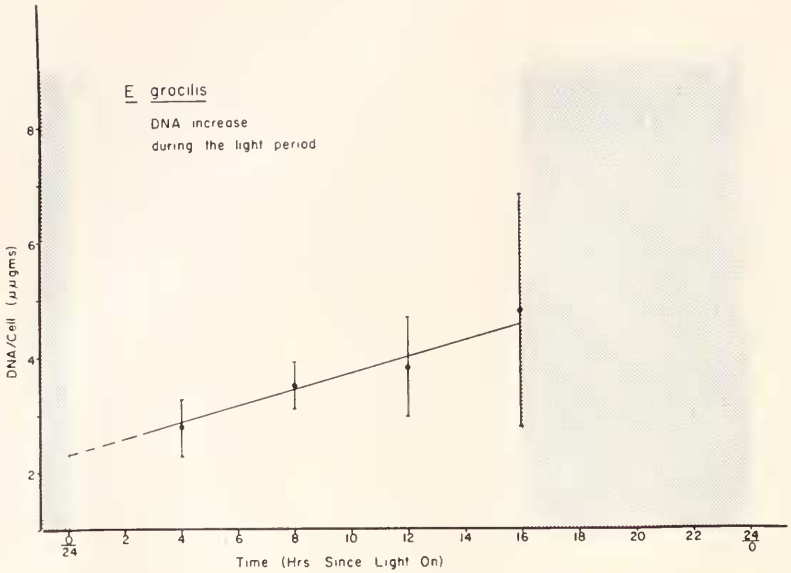

FIGURE 5. DNA content of the average *Euglena* during the light period.

Euglena constitute one to two per cent of the total dry weight (Cook, 1960), and this, too, goes through a slight maximum in the light period.

Table I lists the concentrations of chlorophyll *b* calculated for different ages. While there is considerable error involved in estimates of this pigment by the method used here, the expected two-fold difference in chlorophyll *b* levels should at least

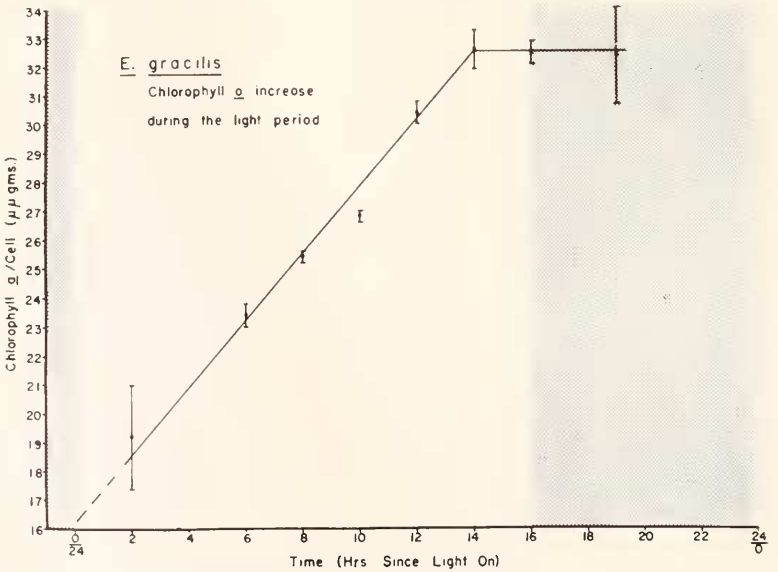

FIGURE 6. Chlorophyll *a* content of the average cell during any given light-dark cycle.

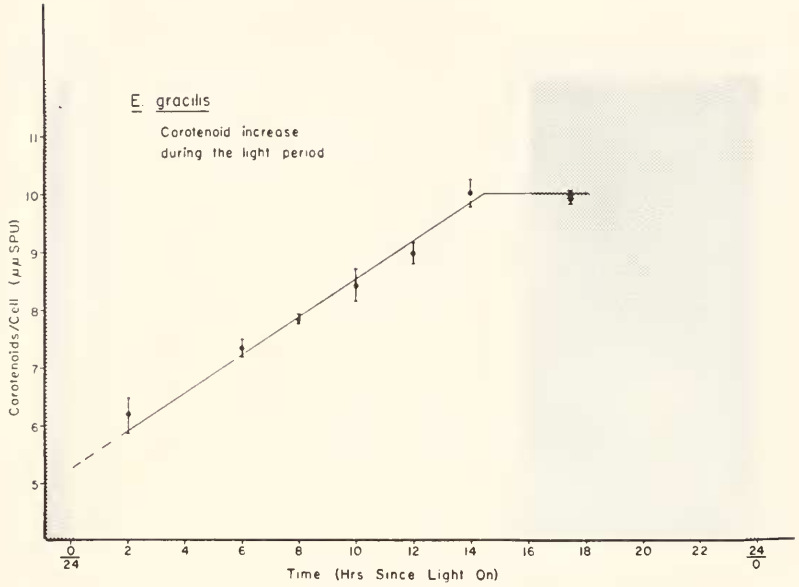

FIGURE 7. Carotenoid synthesis by the average *Euglena*.

be indicated; that is to say, even rough approximations should reveal some tendency toward increasing amounts if chlorophyll *b* is indeed duplicated in the light period. No such differences are seen over the major portion of the light period, however; at the sixth hour, the average cell has 2.60 ± 0.38 μμgrams chlorophyll *b*, and at the onset of the dark period, 2.67 ± 0.24 μμgrams. It is not at all unlikely that chlorophyll *b* is duplicated in the very early portion of the light period. The uncertain role of chlorophyll *b* in the family of photosynthetic pigments need not after all demand a constant ratio of chlorophyll *a*:chlorophyll *b*. Thus, Smith (1949) and Koski (1950) have shown that the biosynthesis of chlorophylls *a* and *b* in etiolated seedlings proceeds at different and apparently unrelated rates. For *Euglena*, a more sensitive analysis than that used here must be adopted before accurate rate measurements of chlorophyll *b* synthesis can be known. The possibility that a continuously changing ratio of photosynthetic pigments occurs over the life cycle of *Euglena* implies a regulation of metabolism which could be of first importance to the over-all balanced growth of the cell.

TABLE I

Chlorophyll b *content of* Euglena gracilis *in synchronous culture, calculated according to the equations of Richards with Thompson (1952). The numbers following "Age" represent the time in hours following onset of the 16-hour light period.*

Age	2	6	8	10	12	14	16	17.5	19
Chlorophyll *b* (μμgm./cell)	1.87 ±.42	2.60 ±.38	2.53 ±.08	2.44 ±.06	2.79 ±.22	3.21 ±.21	2.67 ±.24	2.86 ±.14	3.11 ±.45

Discussion

The energy supply supporting cell division, an event which comes in the dark period in synchronous populations of *Euglena gracilis,* as well as maintenance of the cell in this period, must ultimately be derived from photosynthetic products elaborated in excess of immediate needs for incremental growth, and stored up during the light period against future requirements of the dark period. In point of fact, an estimate of the quantity of this stored energy was the basis leading to the particular light-dark cycle on which *Euglena* is grown (Cook and James, 1960). One might therefore have expected the total dry mass of the average cell to somewhat more than double during active synthesis in the light period. Figure 2 indicates that this is not the case, however. Each parent cell of 1000 $\mu\mu$grams dry weight yields two filial cells of 500 $\mu\mu$grams (assuming equal division), and each of these enters the subsequent light period with just this weight. Thus, parent and filial cells traverse the entire 8-hour dark period with no significant reduction of mass.

Cramer and Myers (1952) have estimated that *Euglena gracilis* consumes twice its own volume of oxygen every hour. While respiratory rates of *Euglena* in synchronous culture have not yet been determined, it is probable that similar rates will be found. With this assumption, and with the further assumption that an RQ of 1 obtains, it can be calculated that the total carbon lost (as CO_2) in the dark period is only 35 $\mu\mu$grams for the average parent and generated filial cells. This is less than 4% of the total dry weight, not likely to be detected by a technique which has a probable error of about 5%.

In most euglenoid flagellates, protein constitutes about 30–40% of the total dry weight (Cook, 1960). In autotrophic strains, photosynthetic pigments contribute about 5%, and the nucleic acids 4 or 5%. Most of the remaining dry mass, some 50%, will be made up of storage products—lipids and paramylum, the characteristic polysaccharide of these cells (Kudo, 1954). Although these storage products were not measured over the life cycle of *Euglena,* it is inferred that their accumulation must complement protein synthesis in such a way that the over-all increase in dry mass, of which these fractions make up the bulk, follows the linear rate shown in Figure 2. The ratio of storage products to dry mass therefore probably passes through a minimum at the same time of the light period as the protein : dry weight ratio exhibits a maximum.

Major synthetic patterns of the average cell in synchronized populations of *Euglena gracilis,* growing autotrophically on a simple, near-basal salt medium at 20° C., are summarized in Figure 8. The ordinate is in relative units, from 1 to 2, the range of increase in these parameters expected for the single cell over its life cycle. The time scale on the abscissa is shifted somewhat from that seen in the earlier graphs, with respect to the light-dark cycle, to more nearly accommodate the actual age of the average cell. This cell has its inception, by division of the parent, some two hours before the onset of the light period, and 24 hours later is itself the parent of two filial cells, an event which occurs at the sixth hour of the subsequent dark period, being preceded by a mitosis which is probably of three or four hours duration (Leedale, 1958). Most of the interphase is spent in the light, where the cell completes its physical growth and duplicates most (if not all) of its principal biochemical constituents. Increase in dry mass is limited to the light period, and follows a linear rate over the entire 16 hours, as does the cell volume (Cook, 1961).

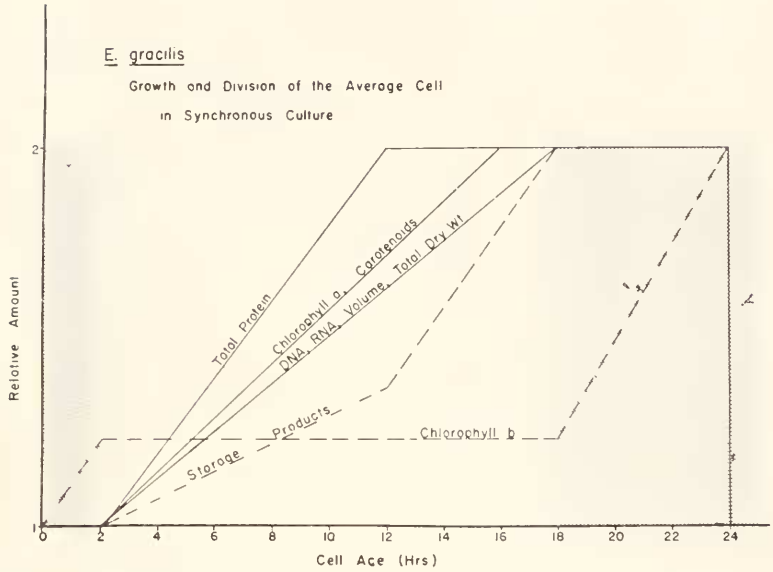

FIGURE 8. Summary of biosynthetic rates of major constituents in the average *Euglena* during any given light-dark cycle. The ordinate shows the relative content, the range of values observed in duplication of the various quantities listed (Figs. 2–7). The broken lines are inferred rates (see text). The average cell is formed by division of a parent about two hours before the onset of the light period (age 0) and divides at about the sixth hour of the subsequent dark period (age 24) to yield two filial cells.

RNA is similarly doubled, and probably also DNA. Protein synthesis, though limited to the light period, is complete by the twelfth or thirteenth hour of the cell's life; storage products, which make up most of the remaining dry mass, are most likely accumulated so as to complement protein synthesis (Fig. 8, broken line). Chlorophyll *a* and the several carotenoids are synthesized only in the light, and are duplicated by the fourteenth hour of this period, when the cell is 16 hours old. Chlorophyll *b* synthesis (Fig. 8, broken line) may occur in the dark period, but the possibility of duplication very early in the light period is not to be excluded. Certainly, most of the cell's growth is confined to the light period.

Division of the average cell, on the other hand, comes in the dark. With well-defined synchronous systems, the average cell is not likely to divide before growth has been completed, and for the present case this must of necessity follow the light period. Moreover, it has been shown that visible light can inhibit cell division in the euglenoid flagellates (Cook, 1960; Leedale, 1959), and division of even mature euglenoid cells can be delayed by light for as much as 20% of their normal generation time. A repetitive cycling of metabolic events, such as the growth patterns described in this paper, coupled with a periodic inhibition of cell division, are doubtless additive in their effect on the continued synchronous divison of *Euglena gracilis*. While photosynthetic cells can of course grow and multiply in continuous light, it is likely that light can be used more effectively for growth (and division) when punctuated periodically by dark periods. Sorokin and Krauss (1959), for example, have reported that *Chlorella* has a higher growth rate with intermittent periods of

dark than in continuous light, and Warburg (1957) has commented that culture of autotrophic cells in continuous light is contrary to conditions demanded by their biochemical evolution. Implicit in all this is the possibility that synthesis of minor cell fractions in synchronized *Euglena,* not necessarily of importance in terms of absolute mass, but yet critical for most effective growth, might well occur with a greater probability in the dark period. It is not unreasonable to imagine that activation energies for such syntheses are reduced in the dark; and culture on a repetitive light-dark cycle would then soon confine these events to the dark period. The varying ratios of the major metabolic constituents of *Euglena,* as seen in Figure 8, further emphasize that metabolic processes need be integrated only with reference to the whole life-cycle of the cell, and not necessarily with any particular portion thereof.

The author wishes to acknowledge the continued encouragement of Dr. T. W. James throughout the course of this work.

Summary

1. When grown autotrophically on an appropriate light-dark regimen, populations of the flagellate *Euglena gracilis* divide synchronously, an approximate doubling of cell number occurring in each dark period.

2. Growth of the average cell during the light period, which is essentially devoid of cell divisions, is described in terms of dry mass, total protein, DNA, RNA, chlorophyll *a,* and the carotenoids. The average *Euglena* duplicates all of these constituents in each light period.

3. Dry mass, volume, RNA, and probably DNA of the average cell increase to a doubling in a linear fashion over the whole of the 16-hour light period.

4. The photosynthetic pigments chlorophyll *a* and the carotenoids (beta-carotene) increase at rates which do not deviate greatly from linearity.

5. Synthesis of total protein is completed 5 or 6 hours before termination of the 16-hour light period. It is inferred that storage products are synthesized at rates complementing protein synthesis in such a way that dry mass increases linearly.

LITERATURE CITED

Abbo, F. E., and A. B. Pardee, 1960. Synthesis of macromolecules in synchronously dividing bacteria. *Biochim. et Biophys. Acta,* **39**: 478–485.

Barner, H. D., and S. S. Cohen, 1956. Synchronization of division of a thymineless mutant of *Escherichia coli. J. Bacteriol.,* **72**: 115–123.

Bernstein, E., 1960. Synchronous division in *Chlamydomonas moewusii. Science,* **131**: 1528–1529.

Cook, J. R., 1960. Light-induced division synchrony of *Euglena gracilis,* and the effects of visible light on related cells. Ph.D. Dissertation. University of California, Los Angeles.

Cook, J. R., 1961. *Euglena gracilis* in synchronous division. I. Dry mass and volume characteristics. *Plant and Cell Physiol.,* **2**: 199–202.

Cook, J. R., and T. W. James, 1960. Light-induced division synchrony in *Euglena gracilis* var. *bacillaris. Exper. Cell Res.,* **21**: 583–589.

Cramer, M., and J. Myers, 1952. Growth and photosynthetic characteristics of *Euglena gracilis. Archiv. f. Mikrobiol.,* **17**: 384–402.

von Denffer, D., 1949. Die planktische Massenkultur pennater Grunddiatomeen. *Archiv. f. Mikrobiol.,* **14**: 159–202.

Fogg, G. E., 1953. The Metabolism of Algae. John Wiley and Sons, Inc., New York.

Goodwin, T. W., and M. Jamikorn, 1954. Studies in carotenogenesis. Some observations on carotenoid synthesis in two varieties of *Euglena gracilis*. *J. Protozool.*, **1**: 216–219.

Hotchkiss, R. D., 1954. Cyclical behavior in pneumococcal growth and transformability occasioned by environmental changes. *Proc. Nat. Acad. Sci.*, **40**: 49–55.

James, T. W., 1954. Quantitative studies on the ribonucleic acid content in *Amoeba proteus* in relation to the nucleus and the division cycle. Ph.D. Dissertation. University of California, Berkeley.

James, T. W., 1960. Controlled division synchrony and growth in protozoan micro-organisms. *In:* Second Conference on Cell Division, New York Acad. Sci., **90**: 550–564.

Koski, V. M., 1950. Chlorophyll formation in seedlings of *Zea mays* L. *Arch. Biochem.*, **29**: 339–343.

Kudo, R. R., 1954. Protozoology. Fourth edition, Charles C Thomas, Publisher, Springfield.

Leedale, G. F., 1958. Mitosis and chromosome numbers in the Euglenineae (Flagellata). *Nature*, **181**: 502–504.

Leedale, G. F., 1959. Periodicity of mitosis and cell division in the Euglenineae. *Biol. Bull.*, **116**: 162–174.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265–275.

Mattern, C. F. T., F. S. Brackett and B. J. Olson, 1957. Determination of number and size of particles by electrical gating: blood cells. *J. Applied Physiol.*, **10**: 56–70.

Mitchison, J. M., and P. M. B. Walker, 1959. RNA synthesis during the cell life cycle of a fission yeast *Schizosaccharomyces pombe*. *Exper. Cell Res.*, **16**: 49–58.

Padilla, G. M., and T. W. James, 1960. Synchronization of cell division in *Astasia longa* on a chemically defined medium. *Exper. Cell Res.*, **20**: 401–415.

Powell, E. D., 1955. Some features of the generation times of individual bacteria. *Biometrika*, **42**: 16–44.

Prescott, D. M., 1959. Variations in the individual generation times of *Tetrahymena geleii* HS. *Exper. Cell Res.*, **16**: 279–284.

Prescott, D. M., 1960. Relation between cell growth and cell division. IV. The synthesis of DNA, RNA, and protein from division to division in *Tetrahymena*. *Exper. Cell Res.*, **19**: 228–238.

Richards, F. A., with T. G. Thompson, 1952. The estimation and characterization of plankton pigments by pigment analysis. II. A spectrophotometric method for the estimation of plankton pigments. *J. Marine Res.*, **11**: 156–172.

Scherbaum, O. H., 1960. Synchronous division of microorganisms. *Ann. Rev. Microbiol.*, **14**: 283–310.

Scherbaum, O., and E. Zeuthen, 1954. Induction of synchronous cell division in mass culture of *Tetrahymena pyriformis*. *Exper. Cell Res.*, **6**: 221–227.

Schmidt, G., and S. J. Thannhauser, 1945. A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. *J. Biol. Chem.*, **161**: 83–89.

Smith, J. H. C., 1949. Processes accompanying chlorophyll formation. *In:* Photosynthesis in Plants, Iowa State College Press, Ames, Iowa.

Sorokin, C., and R. W. Krauss, 1959. Maximum growth rates in synchronized cultures of green algae. *Science*, **129**: 1289.

Summers, L., 1960. The physiological variation of cell and nuclear volumes. Ph.D. Dissertation. University of California, Los Angeles.

Sweeney, B. M., and J. W. Hastings, 1958. Rhythmic cell division in populations of *Gonyaulax polyedra*. *J. Protozool.*, **5**: 217–224.

Tamiya, H., K. Shibata, T. Sasa, T. Iwamura and Y. Morimura, 1953. Effect of diurnally intermittent illumination on the growth and some cellular characteristics of *Chlorella*. *In:* Algal Culture from Laboratory to Pilot Plant (ed. J. S. Burlew), Carnegie Institution of Washington Publication 600, Washington, D. C.

Warburg, O., 1957. Photosynthesis. *Angewandte Chemie*, **69**: 627–634.

Zeuthen, E., 1958. Artificial and induced periodicity in living cells. *In:* Advances in Biological and Medical Physics, vol. VI, Academic Press Inc., Publishers, New York.